

CLAIMS

This listing of claims will replace all prior versions, and listing of claims in the application.

1. (Previously Presented) A process for decreasing the amount of an impurity produced in recombinant production of a growth hormone antagonist polypeptide B-2036 of [SEQ. ID. NO. 1] in genetically modified host cells, the process comprising the step of:

(a) contacting with said impurity under sufficient conditions a mercapto compound to decrease said amount of said impurity, wherein said impurity is a trisulfide isoform of said polypeptide.

2. (Previously Presented) The process of claim 1 further comprising the step of:

(b) growing said host cells to produce said polypeptide, wherein said growing is conducted either before or during said contacting step (a).

3. (Previously Presented) The process of claim 2 further comprising the step of:

(c) purifying said polypeptide to yield a purified polypeptide.

4. (Previously Presented) The process of claim 3 further comprising the step of:

(d) pegylating said purified polypeptide.

5. (Previously Presented) The process of claim 2 wherein said mercapto compound is selected from the group consisting of sulfites, glutathione, beta-mercaptoethanol, dithiothreitol, mercaptoethylamine, dithioerythritol, tris(2-carboxyethyl) phosphine hydrochloride, cysteine, and cysteine in combination with cystine.

6. (Previously Presented) The process of claim 1 wherein said mercapto compound is selected from the group consisting of sulfites, glutathione, beta-

mercaptoethanol, dithiothreitol, mercaptoethylamine, dithioerythritol, tris(2-carboxyethyl) phosphine hydrochloride, cysteine, and cysteine in combination with cystine.

7. (Previously Presented) The process of claim 5 wherein said mercapto compound comprises cysteine or a combination of cysteine and cystine.

8. (Previously Presented) The process of claim 6 wherein said mercapto compound comprises cysteine or a combination of cysteine and cystine.

9. (Previously Presented) The process of claim 7 wherein in said contacting step (a), said trisulfide isoform is contacted with said cysteine or combination of cysteine and cystine for a period of time sufficient to decrease said amount of said trisulfide isoform by at least about 10%.

10. (Previously Presented) The process of claim 9 wherein said period of time is sufficient to decrease said amount of said trisulfide isoform by at least about 50%.

11. (Previously Presented) The process of claim 8 wherein in said contacting step (a), said trisulfide isoform is contacted with said cysteine for a period of time sufficient to decrease said amount of said trisulfide isoform by at least about 10%.

12. (Previously Presented) The process of claim 11 wherein said period of time is sufficient to decrease said amount of said trisulfide isoform by at least about 50%.

13. (Previously Presented) The process of claim 1 wherein said mercapto compound is provided in a buffer.

14. (Previously Presented) The process of claim 2 wherein said mercapto compound is provided in a buffer.

15. (Previously Presented) The process of claim 7 wherein said cysteine or combination of cysteine and cystine is provided in a buffer.

16. (Previously Presented) The process of claim 8 wherein said cysteine or combination of cysteine and cystine is provided in a buffer.

17. (Previously Presented) The process of claim 15 wherein before said contacting step (a), said buffer has an initial combined cysteine and cystine concentration of at least about 0.1 mM.

18. (Previously Presented) The process of claim 16 wherein before said contacting step (a), said buffer has an initial combined cysteine and cystine concentration of at least about 0.1 mM.

19. (Previously Presented) The process of claim 17 wherein said initial combined cysteine and cystine concentration is from about 0.1 mM to about 10 mM.

20. (Previously Presented) The process of claim 18 wherein said initial combined cysteine and cystine concentration is from about 0.1 mM to about 10 mM.

21. (Previously Presented) The process of claim 19 wherein said initial combined cysteine and cystine concentration is from about 1 mM to about 5 mM.

22. (Previously Presented) The process of claim 20 wherein said initial combined cysteine and cystine concentration is from about 1 mM to about 5 mM.

23. (Previously Presented) The process of claim 13 wherein said buffer is selected from the group consisting of Tris, phosphate, HEPES, citric acid, triethylamine, and histidine.

24. (Previously Presented) The process of claim 14 wherein said buffer is selected from the group consisting of Tris, phosphate, HEPES, citric acid, triethylamine, and histidine.

25. (Previously Presented) The process of claim 15 wherein said buffer is selected from the group consisting of Tris, phosphate, HEPES, citric acid, triethylamine, and histidine.

26. (Previously Presented) The process of claim 16 wherein said buffer is selected from the group consisting of Tris, phosphate, HEPES, citric acid, triethylamine, and histidine.

27. (Previously Presented) The process of claim 23 wherein said buffer comprises Tris.

28. (Previously Presented) The process of claim 26 wherein said buffer comprises Tris.

29. (Previously Presented) The process of claim 25 wherein said buffer comprises Tris.

30. (Previously Presented) The process of claim 24 wherein said buffer comprises Tris.

31. (Previously Presented) The process of claim 29 wherein after said contacting step (a) said Tris buffer has a Tris concentration from about 1 mM to about 200 mM.

32. (Previously Presented) The process of claim 28 wherein after said contacting step (a) said Tris buffer has a Tris concentration from about 1 mM to about 200 mM.

33. (Previously Presented) The process of claim 31 wherein said Tris concentration is from about 10 mM to about 50 mM.

34. (Previously Presented) The process of claim 32 wherein said Tris concentration is from about 10 mM to about 50 mM.

35. – 38. (Cancelled)

39. (Previously Presented) The process of claim 26 wherein before said contacting step (a), said buffer has an initial combined cysteine and cystine concentration of at least about 0.1 mM.

40. (Previously Presented) The process of claim 25 wherein before said contacting step (a), said buffer has an initial combined cysteine and cystine concentration of at least about 0.1 mM.

41. (Previously Presented) The process of claim 25 wherein said combination of cysteine and cystine in said buffer and said B-2036 before said contacting step (a) have a molar ratio of moles of combined cysteine and cystine to moles of B-2036 from about 0.5 to about 1000.

42. (Previously Presented) The process of claim 26 wherein said combination of cysteine and cystine in said buffer and said B-2036 and before said

contacting step (a) have a molar ratio of moles of combined cysteine and cystine to moles of B-2036 from about 0.5 to about 1000.

43. (Previously Presented) The process of claim 25 wherein after said contacting step (a) said B-2036 in said buffer has a B-2036 concentration from about 0.1 mg/ml to about 30 mg/ml.

44. (Previously Presented) The process of claim 26 wherein after said contacting step (a) said B-2036 in said buffer has a B-2036 concentration from about 0.1 mg/ml to about 30 mg/ml.

45. (Previously Presented) The process of claim 43 wherein said B-2036 concentration is from about 0.5 mg/ml to about 20 mg/ml.

46. (Previously Presented) The process of claim 44 wherein said B-2036 concentration is from about 0.5 mg/ml to about 20 mg/ml.

47. (Previously Presented) The process of claim 45 wherein said B-2036 concentration is from about 1 mg/ml to about 10 mg/ml.

48. (Previously Presented) The process of claim 46 wherein said B-2036 concentration is from about 1 mg/ml to about 10 mg/ml.

49. (Previously Presented) The process of claim 25 wherein after said contacting step (a) said buffer has a pH from about 6 to about 9.

50. (Previously Presented) The process of claim 26 wherein after said contacting step (a) said buffer has a pH from about 6 to about 9.

51. (Previously Presented) The process of claim 49 wherein said pH is from about 7.5 to about 8.5.

52. (Previously Presented) The process of claim 50 wherein said pH is from about 7.5 to about 8.5.

53. (Previously Presented) The process of claim 25 wherein said buffer and said B-2036 are maintained at a temperature from about 0°C to about 25°C after said contacting step (a).

54. (Previously Presented) The process of claim 26 wherein said buffer and said B-2036 are maintained at a temperature from about 0°C to about 25°C after said contacting step (a).

55. (Previously Presented) The process of claim 53 wherein said temperature is from about 2°C to about 8°C.

56. (Previously Presented) The process of claim 54 wherein said temperature is from about 2°C to about 8°C.

57. (Previously Presented) The process of claim 25 wherein said contacting step (a) is conducted for a time of at least about 30 minutes.

58. (Previously Presented) The process of claim 26 wherein said contacting step (a) is conducted for a time of at least about 30 minutes.

59. (Previously Presented) The process of claim 57 wherein said time is from about 1 hour to about 24 hours.

60. (Previously Presented) The process of claim 58 wherein said time is from about 1 hour to about 24 hours.

61. (Previously Presented) The process of claim 59 wherein said time is from about 1 hour to about 4 hours.

62. (Previously Presented) The process of claim 60 wherein said time is from about 1 hour to about 4 hours.

63. (Previously Presented) The process of claim-25 wherein after said contacting step (a) said B-2036 in said buffer has a volume from about 1 L to about 5000 L.

64. (Previously Presented) The process of claim 26 wherein after said contacting step (a) said B-2036 in said buffer has a volume from about 1 L to about 5000 L.

65. (Previously Presented) The process of 63 wherein said volume is from about 10 L to about 500 L.

66. (Previously Presented) The process of claim 64 wherein said volume is from about 10 L to about 500 L.

67. (Presently Amended) The process of claim 65 wherein said volume is from about 100 L to about 300 L.

68. (Previously Presented) The process of claim 66 wherein said volume is from about 100 L to about 300 L.

69. (Withdrawn and currently amended) A process for decreasing the amount of an impurity produced in recombinant production of a growth hormone antagonist polypeptide in genetically modified host cells containing cellular component(s), the process comprising the step of:

(a) contacting a ~~chelating agent~~ mercapto compound under sufficient conditions with (1) said impurity, (2) said growth hormone antagonist polypeptide, (3) said cellular component(s) and (4) combinations thereof to decrease said amount of said impurity, wherein said impurity is a trisulfide isoform of said polypeptide.

70. (Withdrawn and currently amended) ~~A process for decreasing the amount of an impurity produced in recombinant production of a growth hormone antagonist polypeptide in genetically modified host cells containing cellular component(s), the process~~ The process of claim 69, further comprising the step of:

(a) contacting a metal salt under sufficient conditions with (1) said impurity, (2) said growth hormone antagonist polypeptide, (3) said cellular component(s) and (4) combinations thereof to decrease said amount of said impurity, wherein said impurity is a trisulfide isoform of said polypeptide.

71. (Withdrawn and currently amended) ~~A process for decreasing the amount of an impurity produced in recombinant production of a growth hormone antagonist polypeptide in genetically modified host cells containing cellular component(s), the process~~ The process of claim 69, further comprising the step of:

(a) contacting a chelating agent under sufficient conditions with (1) said impurity, (2) said growth hormone antagonist polypeptide, (3) said cellular component(s) and (4) combinations thereof to decrease said amount of said impurity,

wherein said impurity is a des-phe isoform of said polypeptide.

72. (Withdrawn and currently amended) ~~A process for decreasing the amount of an impurity produced in recombinant production of a growth hormone antagonist polypeptide in genetically modified host cells containing cellular component(s), the process~~ The process of claim 69, further comprising the step of:

(a) contacting a metal salt under sufficient conditions with (1) said impurity, (2) said growth hormone antagonist polypeptide, (3) said cellular component(s) and (4) combinations thereof to decrease said amount of said impurity, wherein said impurity is a des-phe isoform of said polypeptide.

73. (Withdrawn and currently amended) ~~A process for decreasing the amount of an impurity produced in recombinant production of a growth hormone polypeptide in genetically modified host cells containing cellular component(s), the process~~ The process of claim 69, further comprising the step of:

(a) contacting a chelating agent under sufficient conditions with (1) said impurity, (2) said growth hormone polypeptide, (3) said cellular component(s) and (4) combinations thereof to decrease said amount of said impurity, wherein said impurity is a des-phe isoform of said polypeptide.

74. (Withdrawn and currently amended) ~~A process for decreasing the amount of an impurity produced in recombinant production of a growth hormone polypeptide in genetically modified host cells containing cellular component(s), the process~~ The process of claim 69, further comprising the step of:

(a) contacting a metal salt under sufficient conditions with (1) said impurity, (2) said growth hormone polypeptide, (3) said cellular component(s) and (4) combinations thereof to decrease said amount of said impurity, wherein said impurity is a des-phe isoform of said polypeptide.

75. (Withdrawn and currently amended) The process of ~~embodiment~~ claim 73, wherein said contacting step (a) is conducted in the absence of a mercapto compound.

76. (Withdrawn and currently amended) The process of ~~embodiment~~
claim 74, wherein said contacting step (a) ~~further comprises contacting with said metal~~
~~salt in combination with a mercapto compound~~ is conducted in the absence of a
mercapto compound.